Quality Control of Sputum Smears Examination by Re-Reading in a Tuberculosis Screening

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Abstract

Background: Sputum smear examination is the basis of diagnosis of tuberculosis and quality control for the correct diagnosis of tuberculosis is necessary. This study was a method survey and performed with 930 smears with the aim to compare the confidence rate of sputum smear examination reported by one laboratory technician with the findings reported by two technicians in a tuberculosis screening program.

Methods: In this method survey study in Qaemshahr, smears collected from one laboratory were sent to another laboratory for re-examination and duplicate reading and the findings were subsequently compared. Cultures were used as a standard control test.

Results: Comparison showed that the rate of agreement between positive and negative findings of both laboratories was %73.8 and %99.3, respectively. First laboratory reading and its culture results were similar in 89% of cases. There was no significant decrease in the frequency of false negative results of smears which were read twice as compared to those which had been read once only.

Conclusion: The current method of screening patients suspected with tuberculosis, in which all sputum smears are read by a single technician, is accepted as an accurate and reliable method for the diagnosis of tuberculosis.

Keywords: *Double-reading, Tuberculosis screening, Quality control*

Introduction

According to the DALY (Disability Adjusted Life Years) criteria, tuberculosis ranks seventh among the worldwide diseases and it is predicted to maintain this position by the year 2020 (1). At present, around two billion persons are infected with the tuberculosis microbe and around 20 million persons suffer from tuberculosis worldwide. Tuberculosis is one of the most deadly diseases in the young and adults (2). The emergence of AIDS (Acquired Immune Deficiency Syndrome) has further intensified the spread of tuberculosis such that it has led to a re-distribution of tuberculosis in areas which had previously been free of infection. The strategy of the WHO for tuberculosis control is the DOTS (Direct Observational Treatment) method, which is comprised of an effective way for diagnosing and treating this disease (1). Microscopic sputum smear examination is the basis for diagnosing tuberculosis and all patients with cough of ≥ 3 weeks duration should perform three sputum smears for identification of acid fast bacilli (3). Quality control of the slides in diagnostic centers, which is part of the national programs (4) is performed in a number of ways: control through culture (5- 7), double reading of sputum smears (4, 5, 8) and duplicate smear preparation (7). In the study performed in Madagaskar within a three-year period which was based on double reading of smears, it was seen that continuous quality control had profound effect on the diagnosis of tuberculosis and true positive results (4, 9). In a

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study performed in Malavi, the rate of agreement between results of initial reading and that of double reading performed by a second technician was 97% (10). In a study performed in Columbia, on the whole, the rate of agreement by double reading was 96.3% (11) and in Nepal this rate was reported to be 90.2% (12). The rate of false positive and false negative smears was 16.3% and 2.4% in Nepal (12), 1.9% and 0.8% in Brazil (13) and 1.8% and 1.8% in Malavi (10), respectively.

In India unbinding checking showed 95 to 100% agreement Blinded rereading at the tuberculosis center revealed that false negative errors. Were grates among the laboratory technicians (2-7%) than senior tuberculosis Laboratory supervision (0-3%) (14,15). Study in Tanzania percentage of agreement of smear reading between fast reading and reading was 42.9% and 100% for positive and negative slides, respectively (16). Another study in the Brazil four hundred sputum smear microscopies for the diagnosis of tuberculosis were analyzed through double- blind readings by six professionals who usually read/supervise microscopes performed in public health care facilities. The sample was stratified to obtain, at least, a reliability of 90% in the double-blind readings, an alpha error of 5%, and a precision of 3%. The results were analyzed using observed reliability and the kappa index. Thirteen errors (0.27%) were found in the transcription of results. Reliability increased when the three distinct categories of positive results [(Acid- Fast- Bacilli) AFB+, AFB++, and AFB+++] were grouped or when inconclusive results were excluded from the analysis (17).

The aim of this study was to compare the confidence rate of sputum smear examination reported by one laboratory technician with the findings reported by two technicians in a tuberculosis program conducted in Qaemshahr City in Iran during the year 2005.

Materials and Methods

This study was a method survey (Cross sectional descriptive- Analytic) in which all sputum smears collected from the tuberculosis laboratory in Qaem-

shahr City (n= 930 slides) during a period of one year were studied.

In two laboratories, (A, B) as a routine, three sputum specimens were collected from each suspected individual by standardized methods and a slide was prepared for each specimen. Ziel Nelson method was used for staining and then examined for the presence of acid fast bacilli by a technician trained in the field of widespread smear preparation and reading within a suggested time of ten minutes.

In this study, in addition to the preparation and reading of sputum smears by the above method, a culture was also performed for each sputum specimen and used as a gold standard for comparison with smear results. From the onset of study, both technicians in laboratories A and B, prepared and coded the slides and subsequently recorded the results in special forms. The smears were collected from both laboratories on a weekly basis and then redistributed such that the slides derived from laboratory A were reread by the technician in laboratory B and vise versa.

Results were recorded in a special form along with the respective code number and culture results. In this study the technicians were blinded towards the main aim of the study, place where the slides were prepared, and the findings of the first technician. Both technicians were alike in respect to knowledge and work experience. The type of microscopes used, staining methods, type and quality of stain and type of glass slides were also similar. In order to compile the results of the first and second technicians, the "or" method was used which meant that any smear which was reported as positive by either technician was recorded as positive.

Chi-square tests kappa statistic agreement was used for data analysis.

Results

A total of 930 slides were studied, of which 7% and 93% were reported to be positive and negative for tuberculosis by the first technician, and 5.8% and 94.2% were reported as positive and

negative by the second technician, respectively. Comparison of the results derived from the first technician with those of the second technician showed that they were similar in 73.8% of cases for positive findings and in 99.3% of cases for negative findings. Comparison of these results with the respective culture results showed that amongst the smears reported as negative by the first and second technician, 99.3% and 98.9% were found to have negative culture results, respectively (true negative) and 0.7% and 1.1% had positive culture findings, respectively (false negative). Also the first and second technician had 89.2% and 96.3% true positive and 13.8% and 3.7% had false positive findings, respectively (Table 1 and 2). Comparison of smear results of both technicians with culture results showed that 99.9% of the slides which were reported as negative by both technicians, had negative cultures (true negative) and 0.1% had positive cultures (false negative) and out of the 70 slides which were reported as positive by both technicians, 87.1% had positive cultures (true positive) and 12.9% had negative cultures (false positive) (Table 3).

Table 1: Frequency distribution of sputum smear results of first technician according to culture findings

Results of first technician Culture	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Positive	56	86.2	6	0.7	62	6.7
Negative	9	13.8	859	99.3	868	93.3
Total	65	100	865	100	930	100

Table 2: Frequency distribution of sputum smear results of second technician according to culture findings

Results of second technician Culture	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Positive	52	96.3	10	1.1	62	6.7
Negative	2	3.7	866	98.9	868	93.3
Total	54	100	876	100	930	100

Table 3: Frequency distribution of sputum smear results of both technicians according to culture findings

Results of both technicians Culture	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Positive	61	87.1	1	0.1	62	6.7
Negative	9	12.9	659	99.9	868	93.3
Total	70	100	860	100	930	100

Discussion

It is clear that with the coming of the (Human Immune Deficiency Virus/Acquired Immune Deficiency Syndrome) HIV/AIDS pandemic, and the increasing rate of tuberculosis, early and correct diagnosis and prompt treatment of tuberculosis is of special importance in the maintenance of community health. An important factor in tuberculosis screening techniques is the confidence rate and accuracy of the laboratory results.

In this study, results showed that 0.7% of smears which had been reported as negative by the first technician were considered to be positive by the second technician and 1.94% of smears which had been reported as negative by the second technician were considered to be positive by the first technician. Therefore, under diagnostic rate for tuberculosis in Qaemshahr City is around 0.7%-1.94%. Also the level of agreement between the

first and second technician is relatively good (Kappa= 0.873 and Kappa= 0.89, respectively).

A study in Tanzania Lot Quality Assurance Sampling (LQAS) method was used to collect 222 sputum smear slides. A total of 190 morning sputum specimens with corresponding slides were selected for culture. First readings were done by technicians at PDCs (Peripheral Tuberculosis Diagnostic Centers) and thereafter selected slides and specimens were sent to CTRL (Central Reference Tuberculosis Laboratory) for re-examination and culture. Culture results were used as a gold standard. Of 222 slides selected, 214 were suitable for re-examination. Percentage of agreement of smear reading between PDCs and CTRL was 42.9% and 100% for positive and negative e slides, respectively. Measure of agreement (kappa statistic) was 0.5, indicating moderate agreement. Of 190 samples cultured, percentage of agreement between smear reading from PDCs and CTRL was 37% and 88.9% for smear positive and negative slides, respectively. Kappa statistic was 0.3 indicating poor-fair agreements (16).

False negative and false positive findings range from 0.7-1.1% and 3.7-13.8%, respectively and the prevalence of false positive results is more than false negative results.

In India Twelve microscopy centre laboratory technicians examined 41978 direct sputum smears for acid-fast bacilli. Senior Tuberculosis Laboratory Supervisors (STLS) checked all positive smears (4696) and 10% of negative smears (4776) in an unblinded fashion as per Revised National Tuberculosis Control Programme (RNTCP) guidelines. Ten per cent of the positive and negative slides and another 10% of unchecked negative slides were selected systematically for blinded rereading at the Tuberculosis Research Centre (TRC); 422 slides were reread without and with restaining. Unblinded checking by STLS of the smears read by the laboratory technicians yielded 95 to 100% agreement. Blinded rereading at the TRC revealed that false- negative errors were grater among the laboratory technicians (2-7%) than the STLS (0-3%). Restaining and blinded

rereading of slides reduced false-positive errors from 27% to 7% (14, 15).

A study in Brazil showed that the rate of false positive findings was more than false negative findings (1.9% vs. 0.8%) (12). However, studies performed in Kenya and Vietnam showed false negative findings to outnumber false positive findings (5, 7).

In this study, comparison of results reported by two individuals (double reading) with culture results showed that 12.9% and 0.1% had false positive and false negative results, respectively and the level of agreement was high between these results. Takahashi from Nepal reported the rate of agreement between microscopic of sputum smears and culture results to be around 86.5% in 1990 to 88.9% in 1992 (12).

The current study showed that the percentage of true negative results reported by both technicians (double reading) has shown a decline when compared with the findings of the first or second technician. However, these changes are not statistically significant. Also, there is 73.8% and 99.3% agreement between the positive and negative findings of both technicians, respectively and on the whole, the findings of both technicians were similar in 79.4% of cases. These values were therefore lower than those of Leon's study in Columbia (93.9% and 98.7% positive and negative results, respectively) (11) and Mundy's study in Maliwi (96.4% and 97.6% for positive and negative findings, respectively and 97% on the whole) (10). In this way, it can be concluded that the microscopic sputum smear examination in Qaemshahr City can be considered as a reliable method of tuberculosis screening due to its high rate of agreement with the standard test (culture). Also, regarding that the difference between false negative findings reported by one technician as compared to those reported by two technicians is not statistically significant, the current screening system used for tuberculosis suspected individuals, in which the smears are read by one technician, seems to be a reliable and accepted means of screening used in Qaemshahr City.

Acknowledgements

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